

# Appendix 3: Elements of Artificial Flower Test

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Artificial flower experiments are performed with a nucleus (“nuc”) colony (about 4000 workers and a fertile queen) placed in an outdoor flight cage. Three feeding periods are typically included in the test design. The initial feeding is with an untreated (blank) sucrose solution (500 g/kg) delivered in both the artificial flower feeder and a standard feeder placed in the flight cage; the second feeding is treated sucrose solutions; and the third feeding is again, an untreated (blank) sucrose solution. The foraging activity and the learning performances are evaluated using an artificial flower feeder adapted from the experimental device described by Pham and Masson (1985). The feeder consists of six feeding sites arrayed on a circular tray (50 cm diameter). Each artificial flower feeder is a plastic Petri dish containing glass balls (allowing landing of foragers on the feeding sites) and filled with a sucrose solution that is, or is not treated with the test compound. The sucrose solution in each Petri dish is maintained at a constant level, and on each side of the feeding sites an odorant (e.g., pure linalool) is allowed to diffuse. To limit the influence of visual or spatial cues, the artificial feeder is rotated slowly (e.g.,  $\frac{1}{3}$  rpm). The device is placed in front of the hive entrance.

The conditioning (pairing odor/sucrose reward) is conducted for 2 hours on the first day. Testing is then carried out on the following days. The testing device is set with three scented devices with food reward alternating with three unscented devices, without any food reward. The testing device is presented for 5 minutes and then replaced by the conditioning device for 15 minutes, with the odor being again associated with a sucrose solution (treated or untreated). For each observation (every 30 seconds over the 5-minute observation period), the number of forager visits on either the scented sites or the unscented artificial flowers is recorded. After each test, the tray is cleaned with ethanol and the Petri dishes are changed to avoid the deposition of marking scent by the forager bees. The volume of sucrose solution up taken by the foragers is measured.

## REFERENCE

- Pham MH, Masson C. 1985. Analyse par conditionnement associative du mecanisme de la reconnaissance de sources alimentaires par l'abeille. *Bu. Soc. Entomol. Fr.* 90:1216–1223.

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